Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 17 (3): 815–820
https://doi.org/10.15560/17.3.815

PENSOFT

Mucor minutus (Baijal & B.S. Mehrotra) Schipper: a rare mucoralean fungi isolated for the first time in northeastern Brazil

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Abstract

Mucor minutus (Baijal & B.S. Mehrotra) Schipper is described for the first time from northeastern Brazil. The specimen was isolated from the soil of Florestas do Jussará, an upland forest fragment in Pernambuco state. In Brazil, *M. minutus* has previously been isolated only from the state of São Paulo from the soil of the Atlantic Forest. The occurrence of *M. minutus* in Florestas do Jussará is presented and discussed, and a detailed description and illustration of the specimen are presented. This article contributes to the knowledge of mucoralean distribution.

Keywords

Mucoromyceta, Mucoraceae, taxonomy, upland forest

Academic editor: Jadson Bezerra | Received 21 January 2021 | Accepted 10 May 2021 | Published 26 May 2021

Citation: de Melo Alves ALS, de Souza CAF, Cordeiro TRL, de Oliveira RJV, Gurgel LMS, da Cruz MO, Santiago ALCM de A (2021) *Mucor minutus* (Baijal & B.S. Mehrotra) Schipper: a rare mucoralean fungi isolated for the first time in northeastern Brazil. Check List 17 (3): 815–820. https://doi.org/10.15560/17.3.815

Introduction

Mucor Fresen. is the most representative genus within the order Mucorales Fr., family Mucoraceae Fr., and phylum Mucoromycota Doweld, with approximately 91 described species (Wijayawardene et al. 2020). Species of this genus are characterized by forming simple or branched sporangiophores arising directly from the substrate which bear globose and/or subglobose, non-apophysate sporangia, and with a few species producing rhizoids (e.g., *M. luteus* Linnem. Ex Wrzosek and *M. irregularis* Stchigel, Cano, Guarro & E. Álvarez, Benny et al. 2014). Species of this genus are typically saprobes, commonly isolated from the soil, stored grains, and decomposing fruits (Santiago et al. 2013; Lima et al. 2015). However, some taxa are able to cause mucormycosis, as is the case of *M. circinelloides* Tiegh., *M. indicus* Lendn., *M. irregularis*, and *M. lusitanicus* Bruderl. (Hoffmann et al. 2013; Walther et al. 2013; Wagner et al. 2019).

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Mucor minutus (Baijal & B.S. Mehrotra) Schipper may be morphologically differentiated from other species by concomitantly forming long, sympodially and monopodially branched sporangiophores, in addition to small, repeatedly sympodially branched sporangiophores, obovoid to ellipsoidal or infrequently pyriform columellae, as well as subspherical or slightly elipsoidal sporangiospores (Schipper 1975). Unfortunately, knowledge of the ecology and distribution of *Mucor minutus* is limited. So far, this species has been reported from Canada (from soil, ITS sequence is available in GenBank: KU556591: unpublished data), Brazil (from soil: Schoenlein-Crusius et al. 2006), China (substrate unspecified, ITS sequence available in GenBank, MT089979: unpublished data), India (holotype, from soil: Baijal and Mehrotra 1966), Malaysia (from soil: Lee et al. 2012), Nigeria (substrate unspecified, ITS sequence available in GenBank, MN606270: unpublished data), Republic of Mauritius (from soil, ITS sequence available in GenBank, MN871809: unpublished data), South Korea (from soil: Nguyen et al. 2017), Switzerland (from soil in cucumber rhizosphere: Girlanda et al. 2001), and Vietnam (from soil, ITS sequence available in GenBank, EU076933: unpublished data) (Fig. 1A). In Brazil, *M. minutus* was previously isolated from an area of the Atlantic Forest, located in the municipality of Cubatão, state of São Paulo (Schoenlein-Crusius et al. 2006) (Fig. 1B)

During a study on the diversity of Mucorales in an upland forest fragment in Pernambuco, northeastern Brazil, a specimen morphologically similar to *M. minutus* was isolated. The identity of the species was confirmed by sequencing the ITS region of rDNA. Here, we present and discuss the occurrence of *M. minutus*. We also provide the description and illustration this rare species isolated from soil samples in the semiarid region of Brazil.

Methods

Soil collection. Soil samples were collected in January 2018 from Florestas do Jussará, an upland forest area in the semiarid region of Brazil, located in the municipality of Gravatá (08°12′S, 035°32′W), 85.5 km from Recife (Fig. 1B). The climate is tropical with a dry summer, and an average annual temperature of 22 °C. The average annual rainfall is 725 mm, with rains concentrated between March and July (Siqueira-Filho and Machado 2001). Using sterilized spatulas, soil samples up to 5 cm deep were collected from 10 different points, respecting

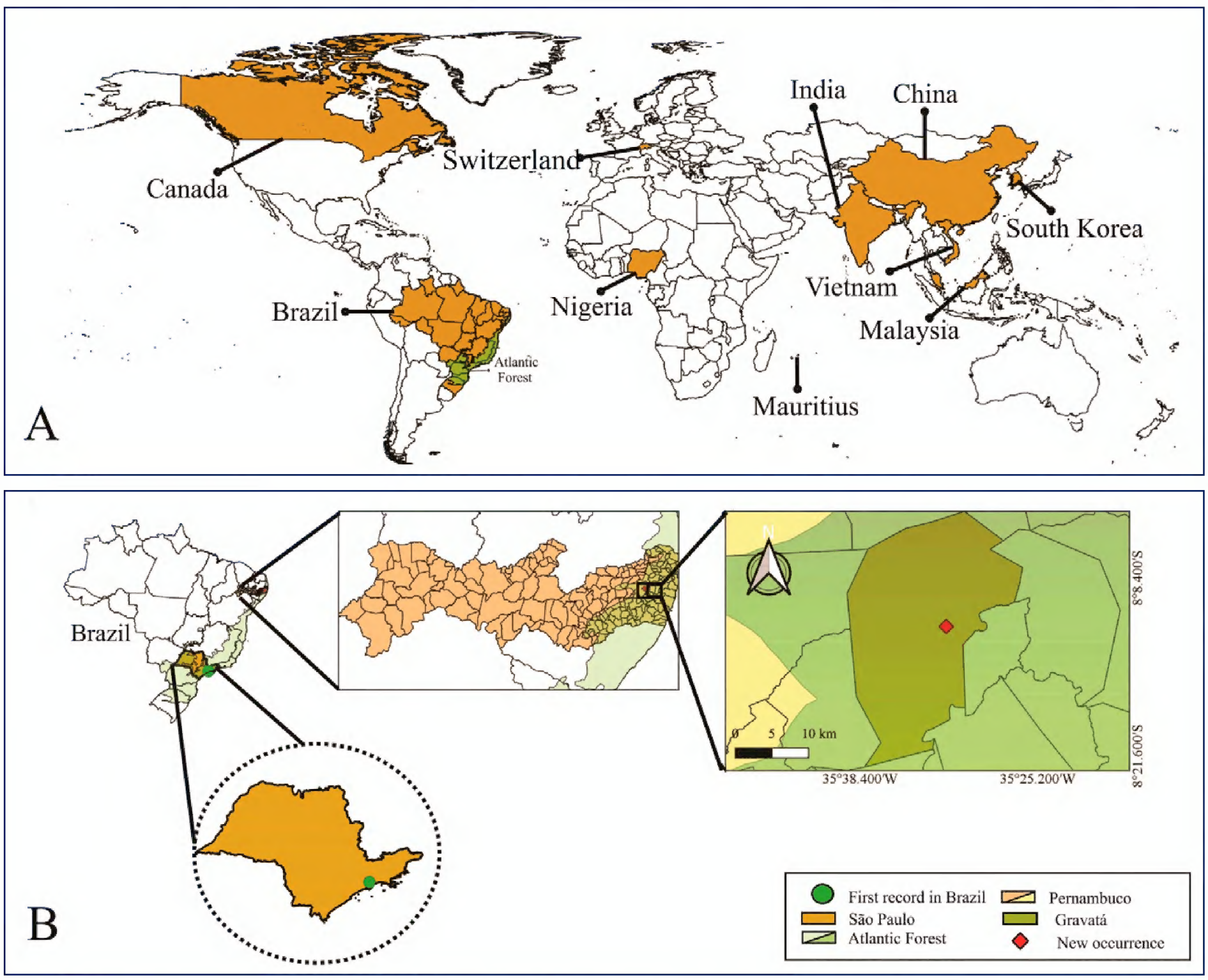

Figure 1. A. Occurrence of *Mucor minutus*. **B.** First occurrence of *M. minutus* (URM 7894) in the semiarid region of Brazil at Reserva Florestas do Jussará, municipality of Gravatá, Pernambuco state.

a minimal distance of 50 m between each on. Samples were placed in clean plastic bags and stored in polystyrene boxes with ice for transport to the laboratory of the Universidade Federal de Pernambuco (UFPE).

Isolation, purification, and identification. A 5 mg sample of soil collected at each one of 10 sampling points was added to Petri dishes containing wheat germ agar culture medium (Benny 2008) plus chloramphenicol (NeoFenicol-Neo Química; 100 mg/L), in triplicate. The plates were stored on a laboratory bench at room temperature (26 ± 2 °C) for 7 days. To purify the specimens, fragments of mycelia were removed from the Petri dishes under a stereomicroscope (Leica EZ4) and transferred to Petri dishes with malt extract agar (MEA) (Benny 2008) plus chloramphenicol (80 mg/L). Fragments of mycelia were transferred to slides with 2% KOH or lactophenol blue and observed using light microscopy (Leica DM500). Each specimen was identified by comparing the macroscopic aspect (colony color and appearance) and microscopic structures (shape and size of the sporangiophores, columellae, sporangia, and sporangiospores), according to the description of Schipper (1975). Seventy-five measurements were made for each fungal structure from plates incubated at 25 °C for 7 days in the dark on MEA. A living culture (URM 7894) is deposited in the culture collection Micoteca URM of the Universidade Federal de Pernambuco.

DNA extraction, PCR, sequencing and phylogenetic analysis. Cultures grown in test tubes containing MEA were incubated at 28 °C for 6 days to obtain fungal biomass. After the material was transferred to 2 mL microtubes with screw caps, 0.5 g of acid-washed glass beads of two different diameters (150–212 µm and 425–600 µm, 1:1; Sigma, USA) were added to each tube. The material was crushed by stirring at high speed in a FastPrep homogenizer. Genomic DNA was extracted as described by de Oliveira et al. (2016). The mycelium was washed with chloroform: isoamyl alcohol (24:1) and then homogenized in 2% cetyltrimethylammonium bromide buffer. The DNA was precipitated in isopropanol, washed with 70% ethanol, and resuspended in 50 µL of ultrapure water. To amplify the ITS region both primers ITS1 and ITS4 where used (White et al. 1990). Polymerase chain reactions were performed as described by de Oliveira et al. (2014). The newly obtained sequence was deposited in the GenBank database (MW436702). The fungal sequences were aligned with ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999). Prior to phylogenetic analysis, the optimal nucleotide replacement model was estimated using TOPALi 2.5 (Milne et al. 2004). Phylogenetic analysis based on the ITS region was performed to reconstruct a phylogenetic tree, under a maximum likelihood (with support estimated by a bootstrap analysis with 1000 replicates) and Bayesian inference (two runs over 2×10^6 generations with a burn-in of 2500) analyses were performed with PhyML 3.0 (Guindon et al. 2010) and MrBayes 3.2.2 (Ronquist et al. 2012), respectively. The phylogenetic tree was visualized using Treeview (Page 1996) and edited using Inkscape v. 1.1 (https://inkscape.org/en/).

Results

Altogether, 273 specimens of Mucorales were isolated from the soil samples, among which one (URM 7894) was morphologically identified as *Mucor minutus*. In the BLASTn analysis, the ITS sequence of URM 7894 showed 100% identity with the sequence of *M. minutus* holotype (CBS 586.67, JN206048), which was grouped together in a same clade, and with other sequences of *M. minutus* in the phylogenetic tree (Fig. 2).

Mucor minutus (Baijal & B.S. Mehrotra) Schipper

Figure 3

Material examined. BRAZIL • Pernambuco, Gravatá, Reserve Florestas do Jussará; 08°12′S, 035°32′W; 739 m a.s.l.; January 2018; Ana L.S Melo Alves leg.; in soil; URM 7894, 1 specimen; GenBank accession no. MW436702 (ITS).

Identification. Colonies initially white, subsequently turned gray, colonized the entire Petri dish (9 cm in diameter and 1.5 cm in height) in five days on MEA at 25 °C. Long and short sporangiophores common. Long sporangiophores hyaline up to 20 µm in diameter, with monopodial and sympodial branches, smooth-walled. Sporangia of the long sporangiophores brownish, globose, up to 160 µm in diameter, slightly equinulated. Columellae of the long sporangiophores hyaline, obovoid, globose, and infrequently conical and flattened,

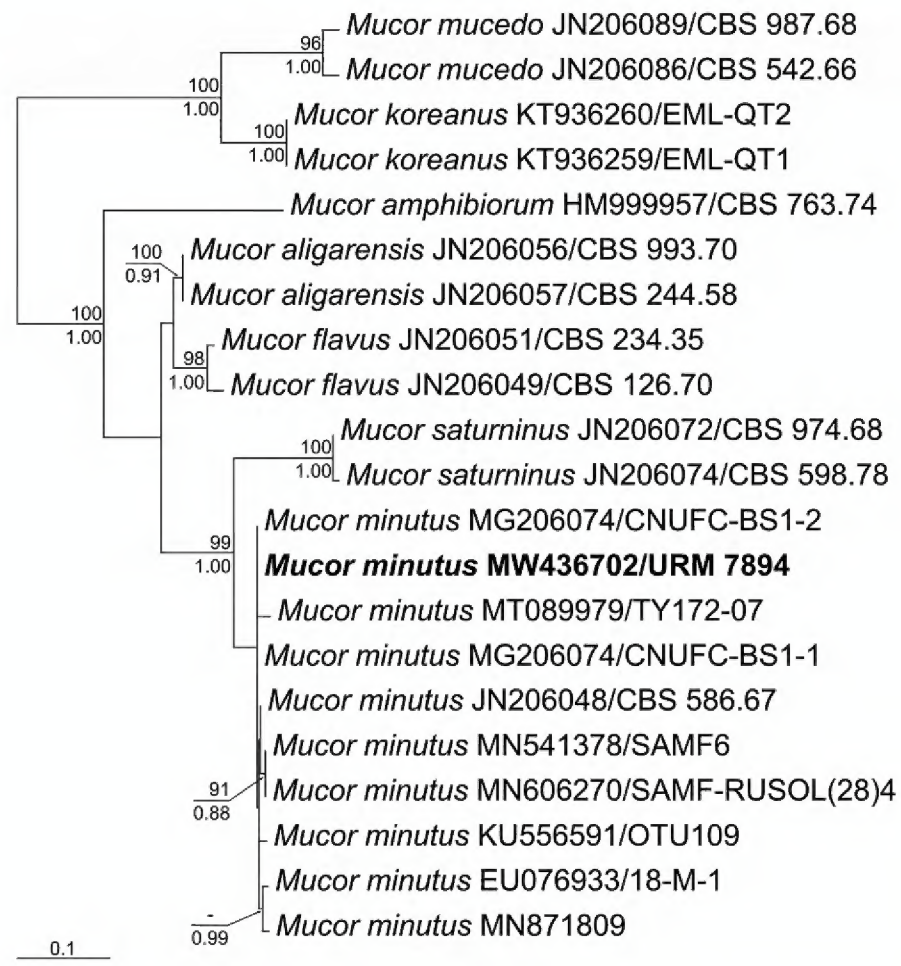

Figure 2. Phylogenetic tree of *Mucor minutus* and related species constructed using the rDNA (ITS) sequences. *Mucor mucedo* and *Mucor koreanus* were used as an outgroup. Supporting values are for maximum likelihood analysis and Bayesian inference. The sequence obtained in this study is in bold.

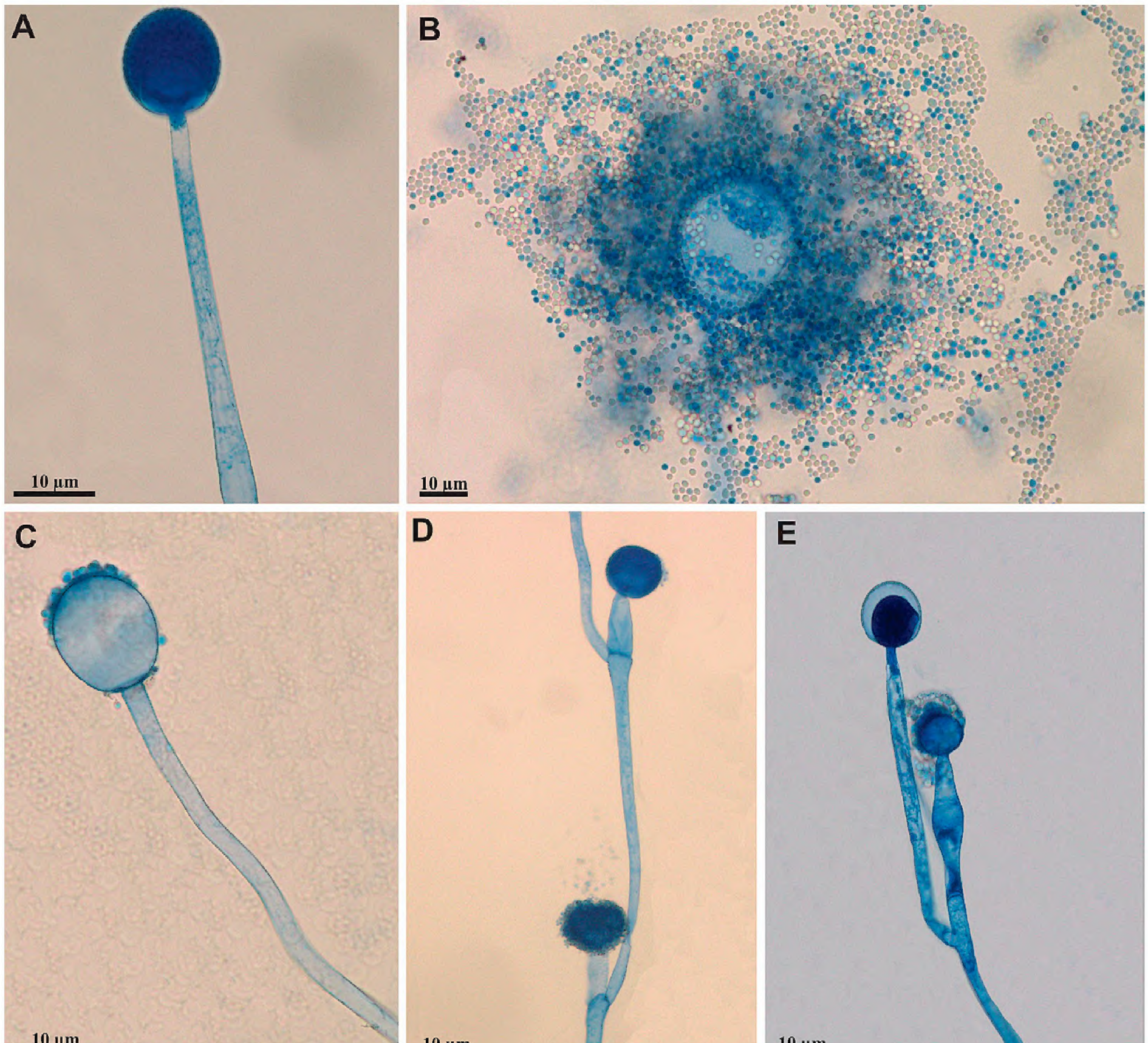

Figure 3. *Mucor minutus* (URM 7894). **A.** Sporangiophore with sporangium. **B.** Sporangium with columella and esporangiospores. **C.** Sporangiophore with columella. **D–F.** Branched sporangiophore. **G.** Sporangiospores.

25–65 (–85) × 25–80 (–95) µm. Short sporangiophores hyaline, repeatedly sympodially branched, up to 12 µm in diameter, smooth-walled. Sporangia of the short sporangiophores hyaline, up to 120 µm. Columellae of the short sporangiophores conical and subglobose, 9.5–19.5 (–36.5) × 7.0–9.5 (–25.0) µm. Sporangiospores hyaline, subglobose to ellipsoid, 2.5–5.0 (–7.2) × 2.3–3.0 (–4.0) µm. Zygospores not observed.

Discussion

We report the first occurrence of the rare *Mucor minutus* (URM 7894) in northeastern Brazil, isolated from soil in an upland forest fragment. This species was first described as a variety of *Mucor griseo-ochraceous* Naumov (as *M. griseo-ochraceous* var. *minutus* Baijal & B.S. Mehrotra) in India (Baijal & B.S. Mehrotra 1966). Nine years later, Schipper (1975) proposed the new combination *M. minutus* (Baijal & B.S. Mehrotra) Schipper. As far as we know, this species has only been reported in Brazil, Canada, China, India, Nigeria, Mauritius, South Korea, Switzerland, and Vietnam. Occurrence data from many of these countries is restricted to ITS sequences deposited in GenBank, with no information as to where specimens were collected. Although published manuscripts reported *M. minutus* from soil in Brazil, India, Malaysia, South Korea, and Switzerland, detailed information on collection sites, including soil type, local vegetation, temperature, and precipitation are missing, except for Brazil. Like other specimens of *Mucor*, *M. minutus* is a probably a soil saprobe, as a parasitism interaction has not yet been reported for this species.

Both morphology and phylogeny show that URM 7894 is *M. minutus*, and the morphological characteristics of this specimen are in good agreement with the description by Schipper (1975). However, some morphological differences were observed between URM 7894 and the ex-type. Long sporangiophores up to 20 µm in diameter were observed in our specimen; these are smaller than those described by Schipper (1975), who described the long sporangiophores as up to 30 µm in diameter. The sporangia of the long sporangiophores of URM 7894 were smaller than those described by Schipper (1975), which are up to 175 µm in diameter. Additionally, URM 7894 exhibits columellae of the long sporangiophores that are obovoid, globose, infrequently conical and flattened, and up to 85 × 95 µm, while Schipper (1975) reported columellae of the long sporangiophores as obovoid to ellipsoid, infrequently pyriform, and up to 135 × 97 µm. The columellae of short sporangiophores described here are conical and subglobose, unlike those reported for the ex-type, which are cylindrical to ellipsoid, some conical to flattened. Nguyen et al. (2017) observed the presence of one to three septa below the columellae, unlike our isolate and the ex-type (Schipper 1975), which have no septum below the columellae.

This manuscript contributes to a better understanding of the distribution of *M. minutus*, a rare species reported from only 10 countries so far. Although most mucoralean species are saprobic and commonly found in soil (Benny et al. 2016), information on the distribution and ecologic niches of these fungi is still scarce (Walther et al. 2019). Unfortunately, there are just a few published manuscripts reporting the occurrence of *M. minutus* (all from soil samples), with little or no information about the biomes where these fungi were found. Thus, new surveys of this species in other substrata, such as dung, decomposing vegetables, and food spoilages in different biomes, may help the understanding on the biology and ecology of this species.

Acknowledgements

This manuscript was financed by the "Mucoromycotina Diversity in the different ecosystems of the Atlantic Forest of Pernambuco" project (FACEPE - First Projects Program PPP/FACEPE/APQ - 0842-2.12/14). We also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the productivity grant awarded to André L. C. Santiago and for the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship awarded to Ana L. Alves.

Authors' Contributions

LMSG collected the material and formatted the plate; CAFS, TRLC, MOC, and RJVO performed the specified methodology; ALSMA and ALCMAS wrote the text and identified the specimen.

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